Clin Exp Immunol 1999; **117**:123–129

Depletion of peritoneal CD5$^+$ B cells has no effect on the course of *Leishmania major* infection in susceptible and resistant mice

B. BABAI, H. LOUZIR, P.-A. CAZENAVE* & K. DELLAGI *Laboratory of Immunology (LI) (LAF 301), Institut Pasteur de Tunis, Tunis-Belvédère, Tunisia, and *Unité d'Immunochimie Analytique, Institut Pasteur, Paris, France*

(Accepted for publication 17 March 1999)

SUMMARY

The mouse peritoneal cavity contains a unique self-renewing population of B cells (B-1) derived from fetal liver precursors and mainly producing polyreactive antibodies. Since B-1 cells are a potential source of IL-10, it has been suggested that these cells may contribute to the susceptibility of BALB/c mice to *Leishmania major* infection by skewing the T helper cell network towards a Th2 phenotype. Accordingly, *L. major* infection of B cell-defective BALB/c Xid mice (lacking B-1 cells) induces less severe disease compared with controls. However, in addition to the lack of B-1 cells, the Xid immune deficiency is characterized by high endogenous interferon-gamma (IFN-γ) production. In the present study, the role of B-1 cells during *L. major* infection was investigated in mice experimentally depleted of peritoneal B-1 cells. Six weeks old C57Bl/6 and BALB/c mice were lethally irradiated and reconstituted with autologous bone marrow which allows systemic depletion of B-1 cells. Untreated BALB/c, C57Bl/6 as well as BALB/c Xid mice were used as controls. After reconstitution, mice were injected with *L. major* amastigotes and progression was followed using clinical, parasitological and immunological criteria. As previously reported, BALB/c Xid mice showed a significant reduction in disease progression. In contrast, despite the dramatic reduction of B-1 cells, B-1-depleted BALB/c mice showed similar or even worse disease progression compared with control BALB/c mice. No differences were found between B-1-depleted or control C57Bl/6 mice. Our data suggest that the B-1 cells do not contribute to the susceptibility of BALB/c mice to *L. major* infection.

Keywords CD5$^+$ B cells *Leishmania major* experimental leishmaniasis

INTRODUCTION

The murine model of infection with *Leishmania major* has been instrumental in the validation *in vivo* of the existence of functional CD4$^+$ T cell subsets and their involvement in determining the outcome of the disease process [1,2]. The clinical course of the infection induced in mice by *L. major* parasites is dependent on the mouse strain used and is strongly associated with the expansion of mutually exclusive T helper cell subsets [3,4]. In parasite-infected, genetically resistant C57Bl/6 mice, a preferential differentiation of interferon-gamma (IFN-γ)-producing T helper 1 (Th1) cells is observed [5]. In contrast, the progressive and fatal disease in mice of the genetically susceptible BALB/c strain is clearly associated with the expansion of IL-4-producing *L. major*-specific T helper 2 (Th2) cells [6,7]. However, there is no consistent view on the role of B cells in the induction of the T cell-mediated anti-Leishmanial immune response. Howard *et al*. [8] demonstrated that antibodies had no effect on the outcome of *L. major* infection. In an earlier study, Sacks *et al*. [9] reported that B cell depletion by intraperitoneal administration of anti-IgM antibodies to new-born BALB/c mice rendered them resistant to subsequent infection with *L. major*, suggesting that B cells inhibit the development of resistance. On the other hand, the same group showed that similarly treated resistant C57Bl/6 mice developed more severe disease [10]. However, Titus *et al*. [11] showed in a later study that the outcome of *L. major* infection in BALB/c mice was not changed by an anti-μ treatment. In fact, in addition to their role in humoral immunity, B cells have other immunoregulatory roles, some of which may be explained by their capacity to produce cytokines [12].

B lymphocytes exist in two subsets, termed B-1 and B-2 [13,14]. In normal humans and mice, B-1 cells are committed to the production of polyreactive natural antibodies, mainly IgM, but also IgG and IgA, which bind a variety of self-antigens (e.g. ssDNA, actin, tubulin, myoglobin) [15–19]. In contrast, conventional B cells (B-2) are mainly involved in the production of antigen-driven antibodies. One of the characteristics of B-1 cells is

Correspondence: Dr Hechmi Louzir, Laboratoire d'Immunologie, Institut Pasteur de Tunis, BP: 74, 1002 Tunis-Belvédère, Tunisia.
E-mail: hechmi.louzir@pasteur.rns.tn

their capacity to produce large amounts of IL-10 in response to different stimuli. Moreover, IL-10 is needed for the development of murine B-1 cells [20].

In a previous study on human visceral leishmaniasis due to *L. infantum*, we have shown that during active disease B-1 cells are expanded and/or activated [21]. Indeed, very high levels of natural autoantibodies were present in the sera of patients with active disease which decreased after treatment. Moreover, $CD5^+$ B cells were increased in the blood of visceral leishmaniasis (VL) patients a few weeks after starting specific therapy.

Several studies on the mouse model have linked B cells (especially B-1) with the development of Th2 dominance, as in mouse models of malaria [22,23], experimental Chagas' disease [24], schistosomiasis [25–27], and leishmaniasis [26,28], and have shown that activated B cells play a crucial role in determining the type of ensuing Th response, in part due to elaboration of IL-10 [29].

Accordingly, in a previous report, Hoerauf *et al*. [30] showed that *L. major* infection of B cell-defective BALB/c Xid mice (lacking B-1 cells) generates a Th1 response associated with less severe disease. However, the Xid immune-deficient mouse strain, in which there is a mutation in the gene encoding the non-receptor protein tyrosine kinase (*Btk*) [31] in addition to the low B-1 cell number [32], is characterized by high endogenous IFN-γ [33].

In the present study, the role of B-1 cells during *L. major* infection was investigated in mice experimentally depleted of peritoneal B-1 cells. BALB/c and C57Bl/6 mice were depleted of B-1 cells by lethal irradiation and autologous bone marrow reconstitution [34]. Surprisingly, treated BALB/c mice, infected 50 days after irradiation with *L. major*, developed a more severe disease compared with controls, despite the complete reconstitution of T and conventional B cells, and the depletion in peritoneal B-1 cells. Our data suggest that B-1 cells do not contribute to the susceptibility of BALB/c mice to *L. major* infection.

MATERIALS AND METHODS

Mice

BALB/c and C57Bl/6 female mice 6–8 weeks old were obtained from Jackson Laboratory (Bar Harbor, ME) and maintained in animal facilities at the Pasteur Institute of Tunis (Tunisia).

The Xid gene-bearing BALB/c female mice 6–8 weeks old were bred at the Pasteur Institute (Unité d'Immunochimie Analytique, Paris, France).

Depletion of peritoneal B-1 cells

Since B-1 cells are maintained by self-renewal in the peritoneal cavity, this population was depleted by lethal irradiation and bone marrow reconstitution as previously described [34]. Briefly, 6-week-old mice were anaesthetized and their hind paws protected with lead. Then they received 8 Gy from a caesium source (^{137}Cs gamma, IBL 437; CIS Bio International, Paris, France) at a rate of 2·7 Gy/min. The mice were maintained in germ-free conditions for at least 3 weeks until reconstitution of haematopoietic cells from the protected bone marrow.

Parasite and infection of mice

A human isolate of *L. major* (Abdou) shown to be the more pathogenic in a comparative study of experimental mice leishmaniasis was used in this study. The parasite was kept virulent by continuous passages in mice. Amastigotes isolated from skin lesions of infected BALB/c mice were purified by differential centrifugation and used to infect mice.

Female mice were injected subcutaneously into the right hind footpad with 2×10^6 amastigotes in a final volume of 50 μl [35]. The development of lesions was monitored by measuring the footpad swelling using a metric caliper.

A frozen-and-thawed preparation of *L. major* promastigotes [36] was used, as leishmanial total antigens (LTA) for *in vitro* lymphocyte stimulation and analysis of DTH reactions. Doses of antigens are expressed in terms of the number of promastigotes from which the antigen was obtained.

DTH

DTH was investigated by injecting LTA (2×10^6) in a final volume of 50 μl into the contralateral non-infected hind footpad. The swelling reaction was measured with a metric caliper at 24 h, 48 h, and 72 h.

Determination of parasite load

For quantification of parasite number, a limiting dilution *in vitro* culture was performed essentially as described [37]. Briefly, infected feet and popliteal draining lymph nodes were homogenized and serial 10-fold dilutions were plated in three replicates at each dilution in 96-well flat-bottomed plates (Nunc, Roskilde, Denmark) containing Schneider's Drosophila medium (Drosophila Schneider Greaces Insect Tissue culture media; GIBCO BRL, Paisley, UK) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 10% heat-inactivated fetal calf serum (FCS). The plates were incubated at 26°C for 10 days and assessed for parasite growth microscopically. Parasite load was expressed as the mean of the negative log parasite last dilution which contained mobile promastigotes.

Cell separation and flow cytometric analysis

Peritoneal cells were obtained from mice by injection of 10 ml of cold RPMI 1640 medium (Life Technologies, Eggenstein, Germany) containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Spleen and lymph nodes were removed and single-cell suspensions were washed three times and resuspended in complete RPMI medium containing 5% FCS.

Peritoneal, splenic and popliteal lymph node cells were characterized using FITC-conjugated rat MoAbs to murine CD4, CD23 and B220, and PE-conjugated rat MoAbs to murine CD8 and CD5 purchased from PharMingen (San Diego, CA). A concentration of 10^6 cells was incubated for 30 min with those MoAbs. Lymphocyte populations were analysed by flow cytometry in an EPICS Profile II flow cytometer (Coulter, Electronics, Hialeah, FL).

Determination of IgG anti-actin antibodies

Rabbit muscle actin was purified according to Spudish & Watt [38]. Screening of sera was done by enzyme immunoassay as follows: 96-well microtitre plates (Nunc) were coated with actin at a final concentration of 1 μg/ml in 100 mM carbonate-bicarbonate buffer pH 9·6. The plates were incubated for 1 h at 37°C, then overnight at 4°C. After washing with PBS containing 0·1% Tween 20 (PBS–T), unreacted binding sites were blocked with 0·5% gelatin in PBS–T for 1 h at 37°C. Wells were washed and filled with 100 μl of sera, diluted 1:100 in PBS–T containing 0·5% gelatin. After 2 h at 37°C, 100 μl of affinity-purified goat anti-mouse IgG (γ-specific) antibody conjugated with peroxidase

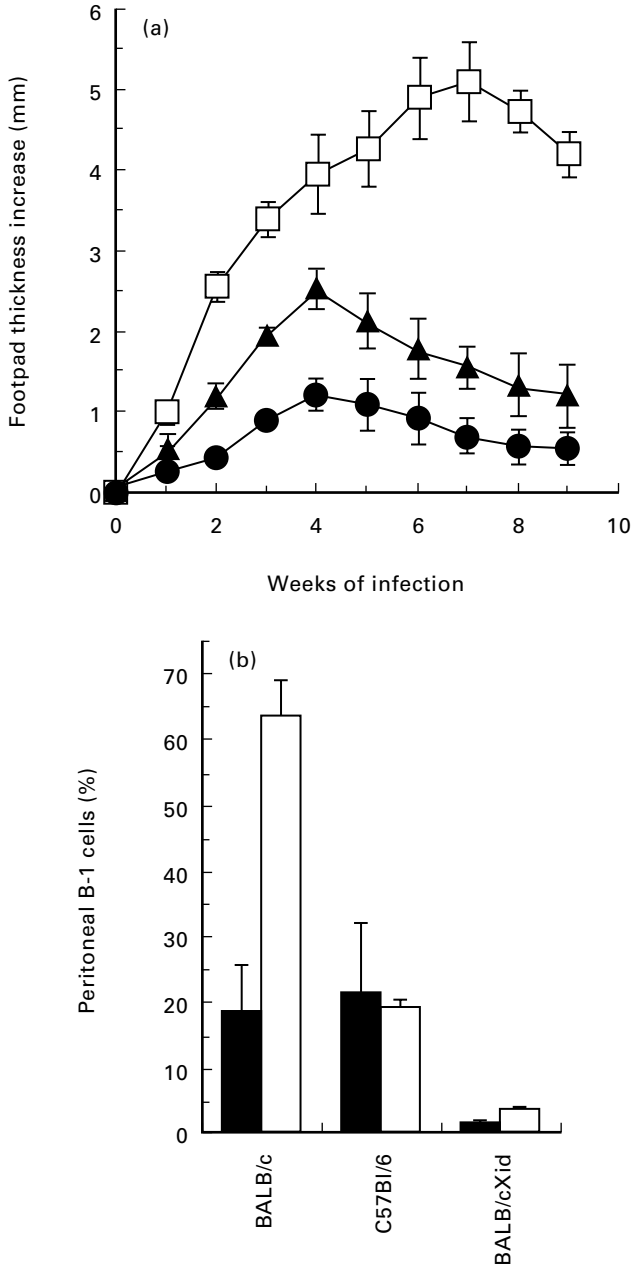

Fig. 1. Lesion progression and proportion of peritoneal $CD5^+$ $B220^+$ B cells in BALB/c (□), C57Bl/6 (●) and BALB/c Xid mice (▲). Groups of five mice were infected in the right hind footpad with 2×10^6 *Leishmania major* amastigotes and lesion progression was monitored as described in Materials and Methods (a). The proportion of peritoneal $CD5^+$ $B220^+$ B cells in those groups of mice before (■) and 9 weeks after *L. major* infection (□) is shown (b). Values given indicate the mean percentage and s.d.

(Immunotech, Marseille, France) were added to the plates and incubated for 1 h at 37°C. The reaction was developed by addition of 1 mg/ml orthophenylene diamine (Sigma, St Louis, MO) prepared in 100 mM citrate buffer pH 5 containing 0·03% hydrogen peroxide and stopped, after 10 min, with 50 μl of 1·5M sulphuric acid. The optical density (OD) was determined in an ELISA reader (Titerteck Muliscan, Helsinki, Finland).

Statistical analysis

Standard deviations of the mean were calculated and the statistical significance of the results was analysed by InStat 2.03 test. $P<0\cdot01$ was considered statistically significant.

RESULTS

Expansion of peritoneal B-1 cells in L. major*-infected BALB/c mice*

The percentage of peritoneal B-1 cells was measured in BALB/c, C57Bl/6 and BALB/c Xid mice before and 9 weeks after *L. major* infection. At this time, as has been previously shown [3], BALB/c mice are unable to control disease progression and develop severe cutaneous lesions. In contrast, C57Bl/6 mice develop localized lesions in the skin that heal spontaneously. As reported previously [30], BALB/c Xid mice showed a significant reduction in disease progression compared with susceptible BALB/c mice (Fig. 1a).

In the resistant C57Bl/6 mice we did not find any change in the proportion of peritoneal $CD5^+$ B cells in infected mice ($19\cdot5\pm2\%$) compared with uninfected controls ($21\cdot5\pm10\cdot6\%$). In contrast, in susceptible *L. major*-infected BALB/c mice we observed a significant increase, more than three-fold, in the proportion of peritoneal B-1 cells compared with controls ($63\cdot7\pm5\cdot3\%$ *versus* $18\cdot5\pm7\cdot2\%$) ($P=0\cdot0035$) (Fig. 1b). As expected, in the BALB/c Xid mice, the proportion of peritoneal B-1 cells did not reach 4%. The percentage of B-1 cells was also measured in spleen and the draining lymph node of BALB/c, C57Bl/6 and BALB/c Xid mice, and each time the levels of B-1 cells ranged between 0·5% and 3% and did not change after *L. major* infection (data not shown).

Increase of anti-actin IgG antibody in the sera of L. major*-infected BALB/c mice*

B-1 cells are committed to the production of polyreactive autoantibodies. Actin constitutes one of the self-antigens frequently recognized by these antibodies [17,19,21]. As an indicator of the level of natural antibodies, anti-actin IgG antibodies were analysed in the sera of *L. major*-infected BALB/c, C57Bl/6, and BALB/c Xid mice obtained 9 weeks after infection and compared with uninfected control groups. Reactions were performed on three experiments using an identically coated plate and results are expressed as OD.

As previously shown in human VL [21], susceptible BALB/c mice showed a marked and significant increase ($P=0\cdot0049$) in anti-actin antibody levels after *L. major* infection (Fig. 2). In contrast, resistant C57Bl/6 and BALB/c Xid mice showed only a modest increase in anti-actin antibody with levels lower than those found in *L. major*-infected BALB/c mice ($P<0\cdot01$).

Depletion of peritoneal B-1 cells increase the susceptibility of BALB/c mice to L. major *infection*

To study the role of B-1 cells during *L. major* infection, we used a previously described method to deplete peritoneal B-1 cells [34] prior to *L. major* inoculation. BALB/c and C57Bl/6 mice were exposed to lethal irradiation with protection of paws in order to allow the reconstitution of the haematopoietic system. Mice were infected 50 days later with *L. major* amastigotes. At this time, levels of B ($B220^+$) and T ($CD4^+$, $CD8^+$) cells in different organs (spleen, lymph nodes and peritoneal cavity) were nearly similar to those of unmanipulated BALB/c or C57Bl/6 control groups, except for peritoneal B-1 ($B220^+$ $CD5^+$) cells, which were significantly

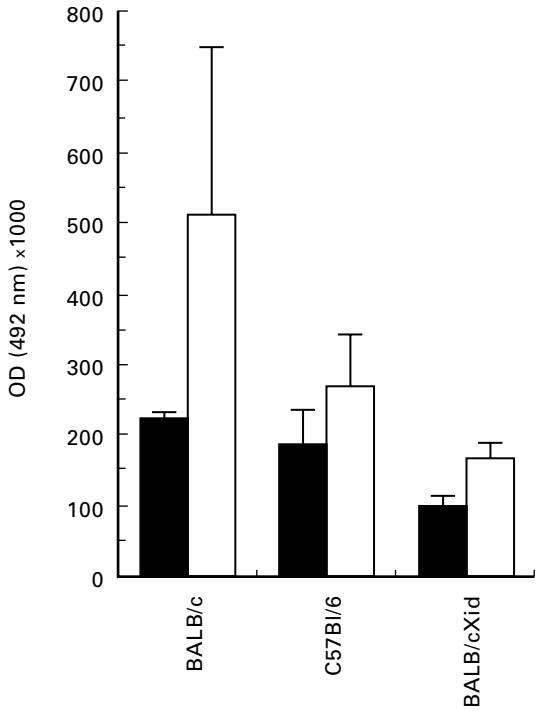

Fig. 2. Serum-IgG anti-actin antibodies in BALB/c, C57Bl/6 or BALB/c Xid mice before (■) and 9 weeks after *Leishmania major* infection (□). Sera from designated mice were diluted 1:100 and tested on the same ELISA plate. Results were expressed as a mean optical density (OD) ± s.d. that were obtained from five individual mice in each group.

lower ($P < 0{\cdot}01$) in irradiated mice compared with controls (Table 1). However, the level of splenic $B220^+$ $CD5^+$ cells seemed unchanged, ranging from 1% to 2%.

Mice were infected with parasite amastigotes and lesion progression was measured during 9 weeks. No differences were found between peritoneal B-1 cell-depleted C57Bl/6 and unmanipulated C57Bl/6 mice: both groups controlled disease progression and the induced lesions healed spontaneously. In contrast, peritoneal B-1 cell-depleted BALB/c mice developed progressive and more severe lesions than those induced in unmanipulated infected BALB/c mice, with a rapid swelling and necrosis of the infected footpad leading to amputation of all infected paws by 8–9 weeks after infection (Fig. 3a).

The levels of B ($B220^+$), T $CD4^+$, T $CD8^+$ and B-1 ($B220^+$ $CD5^+$) cells were analysed in spleen, lymph nodes and peritoneal cavity of the different groups of mice, before and 9 weeks after infection. The B-1-depleted BALB/c mice still had a reduced proportion of peritoneal $CD5^+$ B cells after infection (5%) compared with the unmanipulated infected BALB/c group (64%) (Fig. 3b). The proportion of the different splenic populations of B or T cells did not show any significant differences in the groups of mice before or 9 weeks after *L. major* infection. However, in the lymph node, an increase in the proportions of conventional B cells (with a subsequent decrease in the proportion of T cells) was found in all groups of resistant and susceptible mice after *L. major* infection.

Parasite burdens in the footpad and draining lymph node correlated with measurements of the local lesions. In peritoneal B-1 cell-depleted BALB/c mice, the parasite load in the infected footpad could not be measured because the latter was amputated, but the parasite burden in the draining lymph node, which is also indicative of the dissemination of the parasite, was similar to or little more than that found in unmanipulated BALB/c mice (Fig. 3c). As expected, peritoneal B-1-depleted C57Bl/6 mice had very few parasites in the infected footpad and the parasite burden was similar to that of unmanipulated C57Bl/6 mice.

Increased susceptibility of B-1-depleted BALB/c mice to L. major *infection is associated with the absence of DTH reaction to* Leishmania *antigens*

The cellular immune response was investigated by assessing DTH

Table 1. Proportions of T, B and $CD5^+$ B cells in different lymphoid compartments in controls and B-1-depleted mice*

Lymphoid compartment	Groups of mice ($n = 5$)	$CD4^+$ T cells (%)	$CD8^+$ T cells (%)	$B220^+$ B cells (%)	$CD5^+$ B cells (%)
Spleen	BALB/c	34·5 (± 3)†	19·5 (± 1·8)	39·5 (± 1·7)	0·9 (± 0·3)
	C57Bl/6	20·5 (± 2)	20 (± 1·1)	53 (± 2·9)	2·2 (± 0·6)
	BALB/cXid	30·5 (± 3)	15·5 (± 1·2)	31·5 (± 8·7)	1·5 (± 0·7)
	B-1-depleted BALB/c	29·5 (± 3)	9 (± 1)	45·5 (± 4)	2·5 (± 1·3)
	B-1-depleted C57Bl/6	28·5 (± 3·3)	13 (± 3·3)	53 (± 5·2)	2·8 (± 1·8)
Lymph node	BALB/c	62·5 (± 2)	25·5 (± 2·7)	10·5 (± 0·8)	0·7 (± 0·2)
	C57Bl/6	54 (± 1·1)	31·5 (± 1·3)	13 (± 2·3)	1·1 (± 0·1)
	BALB/cXid	65 (± 1·8)	28·5 (± 1·3)	5 (± 1·3)	0·7 (± 1·7)
	B-1-depleted BALB/c	52 (± 8·3)	21·5 (± 3·9)	23·5 (± 9·2)	1·7 (± 0·3)
	B-1-depleted C57Bl/6	47·5 (± 5·6)	23 (± 7·5)	28 (± 10·7)	2·2 (± 0·5)
Peritoneal cavity	BALB/c	ND	ND	60 (± 8·1)	18·6 (± 7·2)
	C57Bl/6	ND	ND	60 (± 6·7)	21·5 (± 10·6)
	BALB/cXid	ND	ND	30 (± 4·5)	1·8 (± 0·3)
	B-1-depleted BALB/c	ND	ND	44 (± 4)	7·5 (± 1·7)
	B-1-depleted C57Bl/6	ND	ND	54 (± 3·3)	7·1 (± 1)

*Mice were lethally irradiated with protection of paws and reconstituted with autologous bone marrow. Analysis were performed 50 days after irradiation.

†Standard deviation in parentheses.

ND, Not determined.

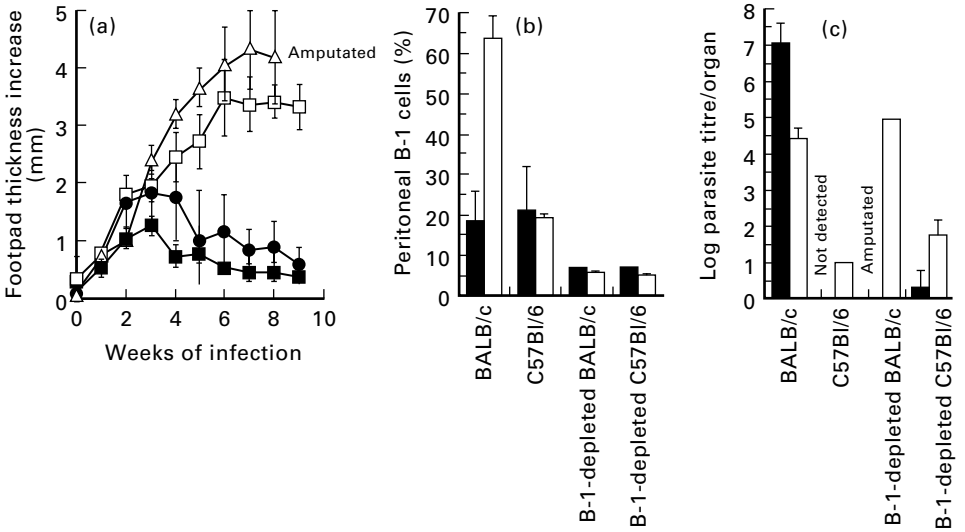

Fig. 3. Course of *Leishmania major* infection in peritoneal B-1-depleted mice. Groups of five mice of B-1-depleted BALB/c (Δ), B-1-depleted C57Bl/6 (■), unmanipulated BALB/c (□), and C57Bl/6 (●) mice were infected with 2×10^6 *L. major* amastigotes and the course of infection was recorded with a metric caliper (a). Footpad measurements represent the mean values (mm) ± s.d. The proportion of peritoneal $CD5^+$ $B220^+$ B cells in the same groups of mice was studied 9 weeks after *L. major* infection (□) compared with similar groups of mice without *L. major* infection (■) (b). Values given indicate mean percentage ± s.d. of double-positive (B-1) cells. Parasite burden at 9 weeks of infection of the same groups of mice (c). Results are expressed as the negative of the mean of the $\log_{10}$ dilution of footpad (■) or draining popliteal lymph node (□) tissue positive for parasite.

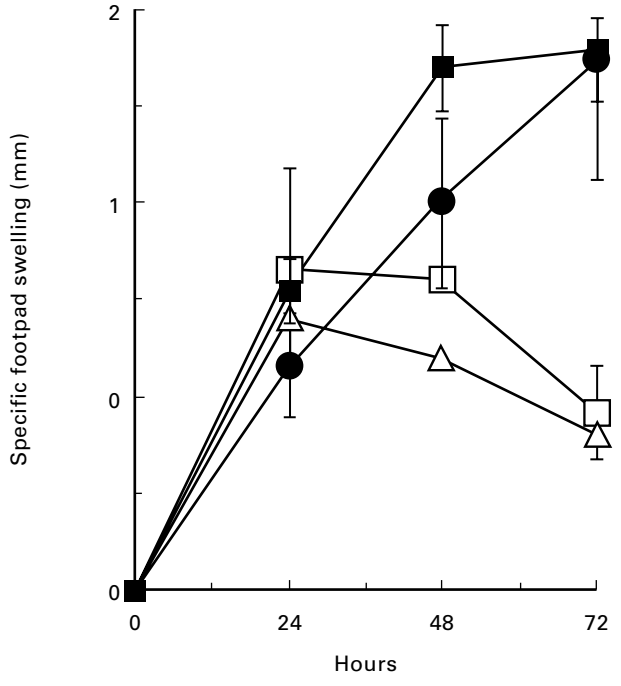

Fig. 4. DTH reactivity of *Leishmania major*-infected mice. At 9 weeks post-infection groups of five B-1-depleted BALB/c (Δ), B-1-depleted C57Bl/6 (■), unmanipulated BALB/c (□) and C57Bl/6 mice (●) were injected with leishmanial total antigens (LTA; 2×10^6) into non-infected hind footpad. The swelling reaction (mm ± s.d.) was measured with a metric caliper at 24 h, 48 h, and 72 h.

reactions. As shown in Fig. 4, either B-1-depleted or unmanipulated C57Bl/6 mice and BALB/c Xid mice developed a positive anti-leishmanial DTH reactivity when tested during the convalescent phase. In contrast, peritoneal B-1-depleted BALB/c mice with persisting lesions had minimal or insignificant DTH responses, as had been found in unmanipulated BALB/c mice.

DISCUSSION

Our results show two aspects: during experimental leishmaniasis, BALB/c mice exhibited a significant increase in peritoneal $CD5^+$ B cell populations and developed high levels of natural autoantibody, suggesting that peritoneal B-1 cells may be activated in parasitized animals. On the other hand, the depletion of peritoneal B-1 cell populations, by lethal irradiation followed by reconstitution with autologous bone marrow in BALB/c mice, did not reduce disease progression despite the dramatic reduction of peritoneal $CD5^+$ B cells. Actually, this treatment worsened significantly the clinical course of the disease.

Cutaneous infection of most inbred mouse strains (e.g. C57Bl/6, C3H and CBA) with the protozoan parasite *L. major* leads to a localized lesion that is contained and resolved primarily by $CD4^+$ Th1 cells [2,39]. Th1 cells secrete the potent macrophage activator IFN-γ, thus stimulating effective killing of this parasite [39,40]. A few inbred mouse strains, such as BALB/c and SWR, fail to control parasite replication. This failure results in progressive lesion development, spread of the parasites to visceral organs and eventually death. Highly susceptible BALB/c mice have been shown to make a strong immune response, but one that is dominated by $CD4^+$ Th2 cells [3], which inhibit macrophage activation by producing IL-4 and IL-10 [29,41]. This sharp delineation of Th1 and Th2 responses has made *L. major* infection a useful model for studying the control of functional differentiation in $CD4^+$ T cells.

The dominant role of cytokines in directing Th effector cell differentiation has been most carefully studied *in vitro* using naive $CD4^+$ T cells from T cell receptor (TCR) $\alpha\beta$ transgenic mice. These experiments have implicated IL-12 in the differentiation of Th1 [42] and IL-4 in the differentiation of Th2 effector cells [43,44].

The requisite role of IL-4 in mediating both Th2 differentiation and susceptibility to *L. major* has directed interest toward the source of the early IL-4 produced *in vivo*. Recent studies have shown that susceptible BALB/c mice, in contrast to resistant strains, exhibited a burst of IL-4 by $V\beta4$ $V\alpha8$ $CD4^+$ T cells in the draining lymph nodes within 16 h after the subcutaneous injection of *L. major* promastigotes [7].

Anti-leishmanial antibodies have no effect on the expression of resistance or susceptibility, as has been demonstrated in transfer experiments using whole serum [8]. The contribution of B cells and antibodies to either the resistance or susceptibility to cutaneous leishmaniasis has been investigated in mouse strains rendered B cell-deficient by intraperitoneal treatment with anti-mouse IgM antisera from birth. This treatment rendered otherwise susceptible BALB/c mice resistant to subsequent infection with *L. major* [9]. However, in a later study evidence for a marginal role of the B cell in the course of experimental leishmaniasis was found by Titus *et al*. [11], and confirmed using severe combined immunodeficient (SCID) mice reconstituted with T cells and different amounts of B cells. The infection of these mice with *L. major* parasites showed that the resistance was strongly T cell-dependent, whereas cotransfer of a fixed number of B cells apparently had no influence on the outcome of the disease [45].

Otherwise, in a previous study of human VL [21], we demonstrated that, during active disease, patients exhibited high levels of natural autoantibodies associated with a significant increase in circulating $CD5^+$ B cells a few weeks after specific therapy, suggesting that these cells are activated during acute disease. Whether this activation contributes to disease progression or is just the consequence of Th2 cytokines remains unknown.

B and B-1 cells are two cell types that are major sources of IL-10 [12,25], and several studies have linked these two types of B cells with the development of Th2 dominance [22–29].

In the present study, we clearly demonstrated that, in BALB/c mice, *L. major* infection leads to high expansion of peritoneal $CD5^+$ B cells associated with a high level of serum anti-actin antibodies. These perturbations are not found in the resistant C57Bl/6 mice. Furthermore, Hoerauf *et al*. [30] showed that BALB/c mice carrying the Xid immunodeficiency had an improved clinical course after *L. major* infection. Here, we found similar results. This does not necessarily mean that the resistance of these mice is due to the absence of B-1 cells. Indeed, in addition to the lack of B-1 cells, Xid immunodeficiency is characterized by high endogenous IFN-γ production [33]. Moreover, it has already been suggested that the resistance of Xid animals to *Trypanosoma cruzi* infection could be due to an elevated endogenous IFN-γ, brought about by their relative deficiency in IL-10. However, deficient levels of IL-10 in B-1 cell-defective BALB/c Xid mice could in turn allow for an exaggerated constitutive expression of IFN-γ. As recently shown by others, high levels of IFN-γ in early development inhibit or eliminate B-1 cells [20].

The resistance of BALB/c Xid mice to *L. major* infection could be due to the genetic defect on tyrosine kinase (*Btk*) and not the consequence of the absence of B-1 cells. This genetic defect could alter the expansion of the lymphoid population, which play a crucial role in Th2 development.

In our study, peritoneal $CD5^+$ B cells were depleted using adult lethal irradiation followed by bone marrow reconstitution. Despite the dramatic decrease of this population in the peritoneal cavity, the depletion seemed not to concern splenic $CD5^+$ B cells. However, this treatment abolishes the capacity of BALB/c mice, when immunized later, to produce $T_{15}{}^+$ idiotype anti-phosphorylcholine antibody [46], which is exclusively produced by $CD5^+$ B cells [13]. These treated BALB/c mice, when infected by *L. major*, did not expand their peritoneal $CD5^+$ B cells, and levels seen in these mice were similar to those obtained with BALB/c Xid mice. However, B-1-depleted BALB/c mice did not control the infection and developed a worse disease compared with control BALB/c mice.

These results suggest that the peritoneal $CD5^+$ B cell expansion found in BALB/c mice after *L. major* infection did not favour the evolution of serious disease and the dissemination of the parasite, but that the expansion of these cells was secondary to disease progression and development of a Th2-type response. In contrast, the expansion of peritoneal $CD5^+$ B cells in susceptible mice confers a certain 'resistance', in so far as BALB/c mice depleted of this population develop a more severe disease compared with unmanipulated BALB/c mice.

ACKNOWLEDGMENTS

This work was supported by Aupelf-Uref Agency. The Laboratory of Immunology of Pasteur Institute of Tunis was also supported during this work by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) (RSG/ID-890266).

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